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Carcinomas of the Respiratory Tract in Hamsters Given Arsenic Trioxide and/or Benzo[a]pyrene by the Pulmonary Route

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The pulmonary carcinogenicity of arsenic trioxide (As) alone and in combination with benzo[a]pyrene (BP) was studied in male Syrian golden hamsters given 15 weekly intratracheal instillations. The animals were divided into four groups: As, BP, As + BP, and controls. At each instillation about 3 mg/kg body wt as arsenic and/or 6 mg/kg body wt of BP was administered. All groups received a carrier dust (charcoal carbon), which increased the lung retention of arsenic, as well as sulfuric acid. Carcinomas of the larynx, trachea, bronchi, or lungs were found in 3, 17, and 25 of 47, 40, and 54 animals examined in the As, BP, and As + BP groups. No respiratory tract carcinomas were found in 53 control animals. The incidence of pulmonary adenomas, papillomas, and adenomatoid lesions was markedly higher in the As group than in the control group ($P < 0.01$). Taken together the data provide strong evidence that arsenic trioxide can induce lung carcinomas. Furthermore, there was some evidence of a positive interaction between arsenic and BP in relation to adenomatous lung tumors, which could be of importance for the synergism between arsenic and smoking observed in smelter workers.

INTRODUCTION

Exposure to inorganic arsenic compounds has been associated with an increased incidence of cancer, predominantly in the respiratory system. Epidemiological investigations of arsenic-exposed copper smelter workers and workers engaged in the production of arsenic-containing pesticides have often revealed positive dose response relationships (Lee and Fraumeni, 1969; Ott *et al.*, 1974; Pinto *et al.*, 1977; Mabuchi *et al.*, 1980). Recently, a multiplicative interaction has been indicated between occupational arsenic exposure and smoking in relation to lung cancer among smelter workers (Pershagen *et al.*, 1981).

Attempts to induce tumors in laboratory animals with inorganic arsenic compounds have mostly failed. Only few studies have, however, involved exposure by the pulmonary route. Ishinishi *et al.* (1977) found indications of a synergism between arsenic trioxide and benzo[a]pyrene for lung cancer in rats exposed via intratracheal instillations. No malignant lung tumors were noted in animals given arsenic trioxide only. In a later study Ishinishi *et al.* (1980) observed one squamous cell carcinoma among a total of 19 rats surviving an exposure of 15 weekly intratracheal instillations of arsenic trioxide. Ivankovic *et al.* (1979) found malignant lung tumors, predominantly adenocarcinomas, in 9 of 15 rats surviving a single intratracheal instillation of a mixture of calcium arsenate, copper sulfate,

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and calcium oxide. The control animals, in which no pulmonary carcinomas appeared, received an intratracheal instillation of 0.9% saline only and consequently a detailed evaluation of the role of arsenic for the carcinogenic effect cannot be made. In a recent study Rudnay and Brozsonyi (1981) were able to increase the frequency of lung tumors in mice by a combined prenatal and postnatal subcutaneous exposure to arsenic trioxide. No detailed histological description of the tumors was given, but apparantly both adenomas and adenocarcinomas occurred.

An increased frequency of chromosomal aberrations has been observed among workers exposed to inorganic arsenic compounds as well as in patients taking arsenic-containing drugs (Petres *et al.*, 1977; Nordenson *et al.*, 1978). In human lymphocytes exposed to arsenite *in vitro* increased rates of chromosomal aberrations and sister chromatid exchanges have been demonstrated (Nordenson *et al.*, 1981, Andersen *et al.*, 1982). Inorganic arsenic is apparently not mutagenic in bacterial tests (Löfroth and Ames, 1978; Rossman *et al.*, 1980), although some conflicting evidence exists (Nishioka, 1975). There are indications, both in bacteria and human cells, that arsenic may interfere with DNA-repair mechanisms, raising the possibility of a cocarcinogenic effect (Jung, 1971; Rossman *et al.*, 1977).

The purpose of this study was to investigate the carcinogenicity of arsenic trioxide in an experimental system which has often been employed succesfully, i.e., intratracheal instillation in hamsters together with a carrier dust. A possible cocarcinogenic role was also tested by simultaneous exposure to benzo[*a*]pyrene (BP) in some animals. Carbon dust was used as carrier in view of its enhancement of the lung retention of BP following an intratracheal instillation (Henry and Kaufman, 1973) and it was shown also to increase the retention of arsenic trioxide. Furthermore the animals were exposed to sulfuric acid because sulfur dioxide and sulfates often occur in smelters together with arsenic.

MATERIALS AND METHODS

Chemicals. BP and charcoal carbon were obtained from Sigma Chemical Co., Saint Louis, Missouri, arsenic trioxide (As_2O_3) and sulfuric acid from Merck, Chemicalien, Darmstadt, Federal Republic of Germany, and sterile 0.9% sodium chloride from Apoteksbolaget, Stockholm, Sweden. The certified purity of BP, arsenic trioxide, and sulfuric acid was 95, 99.5, and 96% respectively. X-ray fluorescence analysis (Sjöstrand, 1977) of the carbon dust showed that the concentrations of antimony, chromium, nickel were below 40 mg/kg. The content of polyaromatic hydrocarbons was also low, i.e., below 1 mg/kg (Stenberg and Alsberg, 1981). The particle size of the carbon dust was determined by microscopic examination. Forty percent of the particles were below 1 μm; 38%, 1.0–1.9 μm; 14%, 2.0–2.9 μm; 5%, 3.0–3.9 μm; and 3% above 4 μm.

Animals. Outbred male Syrian golden hamsters from the Wennergren Institute, Stockholm, Sweden, were used and the animals were 7–9 weeks old at the start of the experiment. They were housed in plastic cages, one animal per cage from the twenty-fifth week of the experiment, at a room temperature of 21°C and relative humidity of 50%

certified not to contain p
animals were given a rat
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Tap water was available

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EXPOSURE DOSES TO ARSEN

Exposure group
As
BP
As + BP
Control

[a] Animals of all exposure g
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[b] At a weight of the animal c

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relative humidity of 50%. The floor of the cages was covered with wood chips, certified not to contain pesticide residues (Anticimex, Sollentuna, Sweden). The animals were given a rabbit and guinea pig maintenance feed (Ewos, Södertälje, Sweden) enriched with sunflower seeds, wheat grain, corn, and dried carrots. Tap water was available *ad libitum*.

Exposure. The animals were exposed by the intratracheal instillation method. The technique was basically the same as used by Saffiotti *et al.* (1968) and has been described previously (Pershagen *et al.*, 1982). The animal was anesthetized by an intraperitoneal injection of a 1% solution of Brietal (Lilly, Indianapolis, Ind.) at a dose of 50 mg/kg body wt. It was then placed on its back on a slanted board hanging in its upper incisors and given via the mouth an instillation with the dust suspension into the lower part of the trachea with a special syringe.

Carcinogenicity test. The animals were divided into four exposure groups: As, BP, As + BP, and controls containing 67, 50, 90, and 68 animals, respectively, at the start of the experiment. Prior to exposure the As and BP dusts were ground together with the carbon dust in a mortar for 15 min and suspended in the saline solution containing 2 mM sulfuric acid. At each of 15 weekly instillations a total volume of about 0.15 ml was given, containing 3 mg of the carbon dust. Throughout the experiment the doses of arsenic and BP were controlled by parallel instillations into glass flasks. The BP dose was chosen from earlier experiments to produce lung cancer in some, but not all, of the animals (Saffiotti *et al.*, 1972a). The arsenic dose was approximately 50% of the LD_{50} dose after a single intratracheal instillation (Pershagen, 1982).

The doses of arsenic and BP in the carcinogenicity test are shown in Table 1. At each instillation a dose of 0.24 mg (as As) was given of arsenic trioxide and 0.45 mg of BP to animals weighing 70–80 g, i.e., about 3 and 6 mg/kg body wt, respectively. The exposure doses were adjusted to the weight of the animal at each instillation.

The animals were observed daily and killed when moribund. Detailed histopathological examinations were performed on coded samples of the larynx, trachea, bronchi, and lungs as well as of other tissues showing macroscopic abnor-

TABLE 1

EXPOSURE DOSES TO ARSENIC TRIOXIDE (As) AND BP AT EACH OF 15 WEEKLY INTRATRACHEAL INSTILLATIONS OF HAMSTERS[a]

Exposure group	Weekly exposure dose[b] (mg ± SD)	
	As_2O_3	BP
As	0.25 ± 0.03	—
BP	—	0.44 ± 0.16
As + BP	0.23 ± 0.07	0.45 ± 0.12
Control	—	—

[a] Animals of all exposure groups received 3 mg carbon particles and 0.03 mg of sulfuric acid at each instillation.

[b] At a weight of the animal of 70–80 g (see text). Doses of arsenic trioxide are expressed as arsenic.

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malities in animals dying after the 15-week exposure period. The tissues were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin–eosin.

Lung retention study. The effect of a carrier dust on the lung retention of arsenic was studied using the same exposure technique and doses of arsenic trioxide, charcoal carbon, and sulfuric acid as in the carcinogenicity test. The exposures were given at four weekly instillations. Five animals were sacrificed immediately following the first instillation, and another five 1 week after two weekly instillations. Four animals were sacrificed 1 week after four instillations and three after another week. The arsenic content in lung was determined in all animals, and the concentration in liver and hair in the animals sacrificed 1 week after two weekly instillations.

A comparison can be made with tissue concentrations of arsenic from a study of a similar design but which did not include the use of a carrier dust for arsenic trioxide (Pershagen *et al.*, 1982). The arsenic dose at each instillation in this experiment was about 0.3 mg. The analysis of arsenic in the tissues were performed using identical procedures in the two studies.

Chemical analysis. The method for arsenic analysis has been described earlier (Pershagen *et al.*, 1982). The tissue samples were digested in an acid mixture and arsines were produced by addition of sodium borohydride. Arsenic was determined by a Perkin Elmer 303 atomic absorption spectrophotometer with deuterium background correction. The content of BP in the glass flasks, instilled simultaneously with the animal exposures, were determined spectrophotometrically at 389 nm (SP6, Pye Unicam Ltd, Cambridge, United Kingdom).

Statistical analysis. The computation of tumor probabilities was performed as suggested by Saffiotti *et al.* (1972b). Significance testing in the carcinogenicity study was made according to Peto *et al.* (1980), with the use of different methods for fatal diseases (e.g., carcinomas of the respiratory tract) and incident diseases (e.g., pathological lung lesions). These methods control for differences in longevity between the treatment groups. The tissue concentrations of arsenic were compared with the Wilcoxon rank sum test and P values of 0.05 or less were regarded as significant.

RESULTS

Table 2 shows the number of animals and survival in the different exposure groups. The mortality during the exposure period differed between the groups. It was highest in the As + BP group (38%) and lowest in the BP group (16%). The deaths usually occurred in connection with the instillations, often at the first instillation of an animal. After the 15-week exposure period the survival was similar in the different groups, with the exception of a tendency toward longer life span among the animals in the As group.

In Table 3 is shown the number of pathological lesions and carcinomas of the larynx, trachea, bronchus, or lung as well as tumors of other organs in animals dying after the fifteenth experimental week in the different exposure groups. Carcinomas of the larynx, trachea, bronchus, or lung were found in 3, 17, and 25 of the 47, 40, and 54 animals examined in the As, BP, and As + BP groups.

SURVIVAL OF MALE HAMSTERS GIVE
TI

Exposure groups	Initial No. of hamsters	15[a]	20	30
As	67	48	48	43
BP	50	42	42	36
As + BP	90	56	53	48
Control	68	53	48	47

[a] Animals dying after the fifteenth w

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Figure 1 depicts the probabi
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a bronchiolar adenocarcinoma
cinoma (Fig. 4). Both the ade

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TABLE 2
SURVIVAL OF MALE HAMSTERS GIVEN 15 WEEKLY INTRATRACHEAL INSTILLATIONS OF ARSENIC TRIOXIDE (As) AND/OR BP

Exposure groups	Initial No. of hamsters	Survivors at experimental week														
		15[a]	20	30	40	50	60	70	80	90	100	110	120	130	140	150
As	67	48	48	43	39	35	31	24	21	17	17	15	7	4	1	0
BP	50	42	42	36	32	27	24	16	12	10	7	6	3	1	0	
As + BP	90	56	53	48	41	37	32	27	22	19	12	8	3	1	0	
Control	68	53	48	47	33	27	25	19	16	13	9	9	7	0		

[a] Animals dying after the fifteenth week were examined histologically.

No malignant tumors of the respiratory tract were observed in 53 animals in the control group. In animals of all four groups tumors in other organs were seen, predominantly lymphosarcomas and sarcomas.

Figure 1 depicts the probability of observation of a carcinoma in the larynx, trachea, bronchus, or lung at the death of an animal in the As, BP, and As + BP groups. The time of occurrence of the tumor as well as the number of animals at risk has been taken into consideration. There is no difference in the occurrence of carcinomas between the BP and As + BP groups. In the As group one carcinoma appeared in an animal dying after 66 weeks and the two other after 102 and 113 weeks, giving a P value of 0.07 (one tailed) by comparison with the control group.

The pathological lesions and carcinomas in the trachea, bronchi, and lungs are further specified in Table 4. In the larynx, trachea, and bronchi the pathological lesions include papillomas and adenomas; in the bronchioli adenomatoid lesions were also seen (cf, Saffiotti *et al.*, 1968). The pathological lesions, not including carcinomas, were more common in each of the exposed groups than in the control group ($P < 0.01$, excluding all animals dying at a higher age than the oldest control animal). Among the pathological lung lesions, adenomas and papillomas were most frequent in the BP and As + BP groups, while adenomatoid lesions dominated among the As-treated animals.

From Table 4 it can also be seen that adenocarcinomas in the bronchi and lungs were the most common malignant tumors in the BP and As + BP groups. Some squamous and mixed adenosquamous carcinomas also appeared in these groups. If adenomatous tumors are taken together (i.e., adenomas, adenocarcinomas, and adenosquamous carcinomas) there seems to be a higher incidence in the As + BP group than in the BP group. This difference is statistically significant for the adenomas ($P = 0.02$) but not for the malignant tumors ($P = 0.2$).

The three carcinomas of the respiratory tract in the As-treated animals were of different morphological types, which showed no unique features in comparison with the carcinomas observed in the BP and As + BP groups. One, a squamous cell carcinoma developed in the larynx (Fig. 2) and the two other in the lung, i.e., a bronchiolar adenocarcinoma (Fig. 3) and bronchiolar small cell anaplastic carcinoma (Fig. 4). Both the adenocarcinoma and the anaplastic carcinoma had a

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TABLE 3

PATHOLOGICAL LESIONS[a] AND CARCINOMAS OF THE LARYNX, TRACHEA, BRONCHUS, OR LUNG AS WELL AS TUMORS OF OTHER ORGANS IN HAMSTERS GIVEN 15 WEEKLY INTRATRACHEAL INSTILLATIONS OF ARSENIC TRIOXIDE (As) AND/OR BENZO[a]PYRENE (BP)

Exposure group	Effective No. of animals[b]	Larynx, trachea, bronchus, or lung			Other organs			
		Carcinoma-bearing animals	Total No. of carcinomas	Lesion-bearing animals[a]	Total No. of lesions[a]	Lymphosarcomas	Sarcomas	Other
As	47	3	3	21	24	5	3	0
BP	40	17	17	23	25	2	2	0
As + BP	54	25	26	28	28	3	5	2[c]
Control	53	0	0	6	7	5	2	0

[a] Adenomas, adenomatoid lesions, and papillomas of the larynx, trachea, bronchus, or lung.

[b] Animals dying after the fifteenth experimental week less those lost due to cannibalism or postmortem changes.

[c] Carcinoma of the kidney and carcinoma of the liver.

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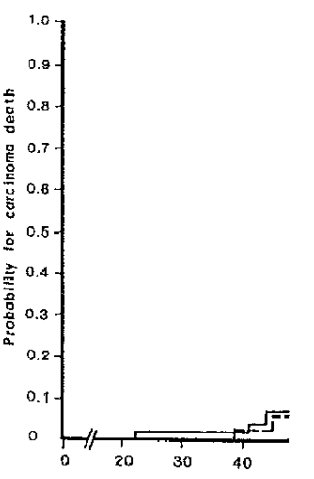

FIG. 1. Probabilities for observatio
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multicentric origin and were located in all lung lobes. The adenocarcinoma was seen macroscopically as greyish white firm nodules a few millimeters in diameter. Microscopically, it was dominated by gland-like structures with mucous production, but solid masses of undifferentiated cells without mucous were also seen. The anaplastic carcinoma, which could be detected only after microscopical examination, had a uniform appearance and consisted of small polygonal cells in solid masses infiltrating bronchiolar and surrounding alveolar tissue.

Figure 5 shows the arsenic concentrations in lung, liver, and hair of hamsters given weekly intratracheal instillations of arsenic trioxide with or without carbon dust. In animals sacrificed 1 week after two weekly instillations the lung concentrations are higher in animals receiving arsenic trioxide with the carbon particles (mean: 5.9 mg As/kg) than without the carrier dust (mean: 0.81 mg/kg). On the other hand, the content of arsenic in liver and hair (mean: 0.3 and 4 mg/kg, respectively) was similar in the two groups. The high arsenic concentrations in lung were further sustained 1 and 2 weeks after four weekly instillations in the animals treated with arsenic and carbon.

DISCUSSION

In spite of strong epidemiological evidence on the pulmonary carcinogenicity of inorganic arsenic among smelter workers, conclusive evidence from experimental studies is lacking (Pershagen, 1981). In the present study arsenic trioxide, which is the most common arsenic compound in smelters, has for the first time been tested in intratracheal instillations to hamsters together with a carrier dust. Three carcinomas of the respiratory tract occurred among the arsenic-treated animals and none in the control group treated with the carrier dust only. Although

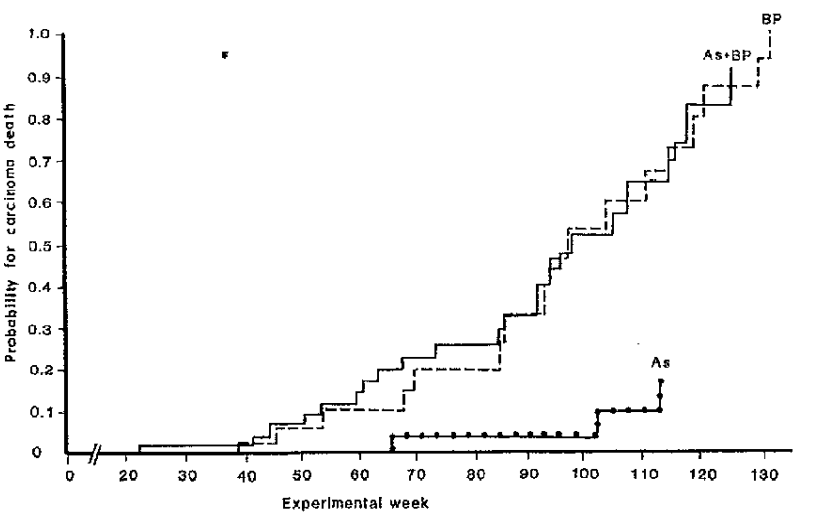

FIG. 1. Probabilities for observation of a carcinoma in the respiratory tract at death of hamsters given intratracheal instillations of arsenic trioxide and/or BP.

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TABLE 4
RESPIRATORY TRACT LESIONS IN HAMSTERS GIVEN 15 WEEKLY INTRATRACHEAL INSTILLATIONS OF ARSENIC TRIOXIDE (As) AND/OR BENZO[*a*]PYRENE (BP)[a]

	Exposure group			
	As	BP	As + BP	Control
Larynx and trachea				
Papilloma	4	11	6	3
Adenoma	1	1	1	0
Carcinoma, squamous cell	1	1	1	0
Carcinoma, anaplastic	0	0	1	0
Bronchi and lungs				
Papilloma	2	1	0	1
Adenomatoid lesions	16	9	9	2
Adenoma	1	3	12	1
Carcinoma, squamous cell	0	5	4	0
Carcinoma, adenocarcinoma	1	9	16	0
Carcinoma, adenosquamous	0	1	4	0
Carcinoma, anaplastic	1	1	0	0

[a] In a few animals two lesions occurred simultaneously, e.g., papilloma of the trachea and adenocarcinoma of the bronchus and lung (cf, Table 3).

these carcinomas do not constitute a statistically significant increase, the higher incidence ($P < 0.01$) of papillomas, adenomas, and adenomatoid lesions in the arsenic-treated animals supports the conclusion that the carcinomas were not a chance finding. The number of these lesions is generally increased in groups of hamsters exposed to carcinogens giving rise to an increased frequency of malignant lung tumors (cf, BP and As + BP exposure groups in this study; Saffiotti *et al.*, 1972a, b; Henry *et al.*, 1973; Stenbäck and Rowland, 1979).

A further support for the findings comes from the fact that no carcinomas in the respiratory tract were seen in a group of 49 male hamsters from the same colony as the animals in the carcinogenicity test, treated identically to the hamsters in the control group (Pershagen, 1982). The animals were exposed 1 year earlier than the animals in the carcinogenicity test and were followed during their whole life span with detailed histopathological examination of death. If these hamsters are also included in the analysis the three carcinomas in the group of arsenic-treated animals become statistically significant ($P \approx 0.01$, one-tailed test). It should be noted that the difference in survival between the arsenic animals and the control animals is taken into consideration in the computation of the P value (cf, Peto *et al.*, 1980) and that none of the lung carcinomas in the arsenic-treated group appeared in an animal dying at a higher age than the oldest animal in the control group or in the group given the same treatment as the control group 1 year earlier.

The retention time in the lungs of BP is of importance for the carcinogenic response (Henry and Kaufman, 1973; Henry *et al.*, 1973). Increased pulmonary retention of BP may explain the potentiating effects by various metal dusts on BP carcinogenieity (cf, Nordberg and Andersen, 1981). The high tumorigenic

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ATRACHEAL INSTILLATIONS OF
IE (BP)[a]

e group	
As + BP	Control
6	3
1	0
1	0
1	0
0	1
9	2
12	1
4	0
16	0
4	0
0	0

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FIG. 2. Squamous cell carcinoma (long arrows) of the larynx in a hamster given 15 weekly intratracheal instillations of arsenic trioxide. Short arrows indicate pieces of cartilage (40×).

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FIG. 3. Adenocarcinoma of the lung with a varying degree of differentiation in a hamster given 15 weekly intratracheal instillations of arsenic trioxide

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FIG. 3. Adenocarcinoma of the lung with a varying degree of differentiation in a hamster given 15 weekly intratracheal instillations of arsenic trioxide (100×).

FIG. 4. Small cell anaplastic carcinoma of the lung in a hamster given 15 weekly intratracheal instillations of arsenic trixoide. Arrows indicate bronchiolar wall. In the left-hand bottom corner a larger magnification of a piece of the bronchiolar wall (arrow) shows the appearance of the carcinoma cells (100× and 250×).

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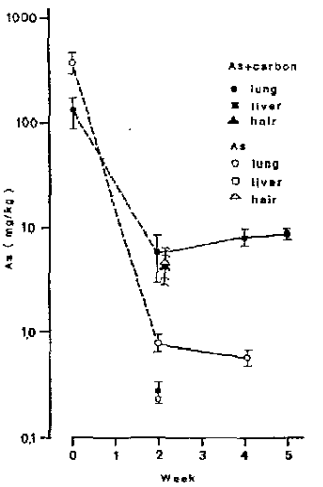

FIG. 5. Arsenic concentration in lung, liver, and hair of hamsters given weekly intratracheal instillations of arsenic trioxide with or without carbon dust. (Bars indicate ± 1 SD.) Animals at Week 0 were killed immediately following the first instillation; other animals were killed 1 or 2 weeks after an instillation (see text).

activity of chromium and nickel compounds of low solubility in relation to soluble forms (Norseth, 1978), indicates that the lung retention may be a crucial factor also for carcinogenic metals. It is of interest that high concentrations of several metals, as well as of arsenic, have been found in the lungs of dead smelter workers many years after cessation of occupational exposure (Brune *et al.*, 1980). This group of smelter workers also had a high risk of lung cancer, which was related to the arsenic exposure (Axelson *et al.*, 1978; Pershagen *et al.*, 1981). In the present study the lung retention of arsenic was enhanced by the use of a carrier dust (charcoal carbon), which may have influenced the cancer response. Arsenic trioxide given without a carrier dust is rapidly cleared from the lung in relation to some arsenic compounds of low solubility also occurring in the occupational environment, i.e., arsenic trisulfide and calcium arsenate (Inamasu *et al.*, 1982; Pershagen *et al.*, 1982). The study by Ivankovic *et al.* (1979), discussed in the Introduction, indicates that calcium arsenate might be a potent pulmonary carcinogen. Further carcinogenicity tests in laboratory animals exposed via the pulmonary route should be performed with arsenic compounds of low solubility.

There are indications of a positive interaction between BP and SO_2 for the induction of lung carcinomas in experimental animals (Laskin *et al.*, 1970). In the present study sulfuric acid was given at the instillations of all groups and it cannot be excluded that it modified the response to both arsenic trioxide and BP.

In a preliminary study of rats exposed at 15 weekly intratracheal instillations Ishinishi *et al.* (1977), found indications of a synergism between arsenic trioxide, as well as two arsenic containing dusts, and BP for the development of pulmonary

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carcinomas. Only adenomatous tumors were found in animals receiving the arsenic dusts without BP. In the groups given BP as well as one of the arsenic dusts the majority of the tumors were squamous cell carcinomas. The small number of animals and a high mortality during the exposure period, also in the control group, makes the interpretation of the results difficult. In other experimental systems no signs of a positive interaction between inorganic arsenic and polyaromatic hydrocarbons have been detected, i.e., by oral administration or skin application of arsenic trioxide, potassium arsenite, and sodium arsenate combined with skin painting with dimethylbenzanthracene (Baroni *et al.*, 1963; Boutwell, 1963). In our study simultaneous exposure to arsenic trioxide and BP did not result in an increased incidence of carcinomas in the respiratory tract. If, however, adenomatous tumors only are considered there seemed to be a positive interaction between arsenic and BP. In this context it is worth noting that the lung tumors observed in the previously mentioned carcinogenicity tests with arsenic trioxide and calcium arsenate were predominantly adenomatous (Ivankovic *et al.*, 1979; Rudnay and Borzsonyi, 1981).

In view of these experimental findings it is of particular interest that a predominance of adenocarcinomas has been found among arsenic-exposed smelter workers with lung cancer (Wicks *et al.*, 1981). An increased proportion of pulmonary adenocarcinomas was also indicated in a similar group of workers (Newman *et al.*, 1976) but not in other arsenic-exposed smelter workers (Axelson *et al.*, 1978). The numbers were too small in these two studies to permit any firm conclusions.

A multiplicative interaction between arsenic exposure and smoking in relation to lung cancer has recently been found among smelter workers (Pershagen *et al.*, 1981). No detailed histopathological analysis of these carcinomas has yet been performed. It is possible that an interaction between arsenic and polyaromatic hydrocarbons or other tumor initiators may have contributed to the findings in the smelter workers. This may be mediated by the effect of arsenic on DNA repair, which has been indicated in different experimental systems (Jung, 1971; Rossman *et al.*, 1977). It would be of interest to further elucidate the histological picture of lung carcinomas among different groups of arsenic-exposed workers.

In conclusion the results of this study provide strong evidence that arsenic trioxide can induce lung carcinomas. The use of a carrier dust enhanced the lung retention of arsenic, which may have increased the tumor response. Sulfuric acid was also administered and might have played a role. There was some evidence of a positive interaction between arsenic trioxide and BP in relation to adenomatous lung tumors which suggests that this mechanism may be of importance for the synergism between arsenic exposure and smoking in smelter workers.

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Source: https://www.industrydocuments.ucsf.edu/docs/jgxk0001

REFERENCES

Andersen, O., Wulf, H. C., Rönne, M., and Nordberg, G. F. (1982). Effects of metals on sister chromatid exchange in human lymphocytes and chinese hamster V79-E cells. *ILO Occupat. Safety Health Ser.* **46**, 491–501.

Axelson, O., Dahlgren, E., Jansson, C-D., and Rehnlund, S. O. (1978). Arsenic exposure and mortality: A Case-referent study from a Swedish copper smelter. *Brit. J. Ind. Med.* **35**, 8–15.

Baroni, C., van Esch, G. J., and Saffiotti, U. (1963). Carcinogenesis tests of two inorganic arsenicals. *Arch. Environ. Health* **7**, 668–674.

Boutwell, R. K. (1963). A carcinogenicity evaluation of potassium arsenite and arsanilic acid. *J. Agric. Food Chem.* **11**, 381–385.

Brune, D., Nordberg, G., and Wester, P. (1980). Distribution of 23 elements in the kidney, liver and lungs of workers from a smeltery and refinery in north Sweden exposed to a number of elements and a control group. *Sci. Total Environ.* **16**, 13–35.

Henry, M. C., and Kaufman, D. G. (1973). Clearance of benzo[*a*]pyrene from hamster lungs after administration of coated particles. *J. Natl. Cancer Inst.* **51**, 1961–1964.

Henry, M. C., Port, C. D., Bates, R. R., and Kaufman, D. G. (1973). Respiratory tract tumors in hamsters induced by benzopyrene. *Cancer Res.* **33**, 1585–1592.

Inamasu, T., Hisanaga, A., and Ishinishi, N. (1982). Comparison of arsenic trioxide and calcium arsenate retention in the rat lung after intratracheal instillation. *Toxicol. Lett.* **12**, 1–5.

Ishinishi, N., Kodama, Y., Nobutomo, K., and Hisanaga, A. (1977). Preliminary experimental study on carcinogenicity of arsenic trioxide in rat lung. *Environ. Health Perspect.* **19**, 191–196.

Ishinishi, N., Mizunoe, M., Inamasu, T., and Hisanaga, A. (1980). Experimental study on carcinogenicity of beryllium oxide and arsenic trioxide to the lung of rats by an intratracheal instillation. *Fukuoka Acta Med.* **71**, 19–26.

Ivankovic, S., Eisenbrand, G., and Preussmann, R. (1979). Lung carcinoma induction in BD rats after single intratracheal instillation of an arsenic-containing pesticide mixture formerly used in vineyards. *Int. J. Cancer* **24**, 786–789.

Jung, E. (1971). Molekularbiologische Untersuchungen zur chronischen Arsenvergiftung. *Z. Haupt. Geschlechtskr.* **46**, 35–36.

Laskin, S., Kuschner, M., and Drew, R. (1970). Studies in pulmonary carcinogenesis. *In* "Inhalation Carcinogenesis" (M. Hanna, P. Nettesheim, and J. Gilbert, Eds.), pp. 321–351. AEC Symposium Series 18. U.S. Atomic Energy Commission, Oak Ridge, Tennessee.

Lee, A. M., and Fraumeni, J. F., Jr. (1969). Arsenic and respiratory cancer in man: An occupational study. *J. Natl. Cancer Inst.* **42**, 1045–1052.

Löfroth, G., and Ames, B. (1978). Mutagenicity of inorganic compounds in Salmonella typhimurium; Arsenic, chromium and selenium. *Mutat. Res.* **53**, 65–66.

Mabuchi, K., Lilienfeld, A., and Snell, L. (1980). Cancer and occupational exposure to arsenic: A study of pesticide workers. *Prev. Med.* **9**, 51–77.

Newman, J., Archer, V., Saccomano, G., Kuschner, M., Auerbach, O., Grondahl, R., and Wilson, J. (1976). Histologic types of bronchogenic carcinoma among members of copper-mining and smelter communities. *Ann. N.Y. Acad. Sci.* **271**, 260–268.

Nishioka, H. (1975). Mutagenic activities of metal compounds in bacteria. *Mutat. Res.* **31**, 185–189.

Nordberg, G. F., and Andersen, O. (1981). Metal interactions in carcinogenesis. *Environ. Health Perspect.* **40**, 65–81.

Nordenson, I., Beckman, G., Beckman, L., and Nordström, S. (1978). Occupational and environmental risks in and around a smelter in northern Sweden. II. Chromosomal aberrations in workers exposed to arsenic. *Hereditas* **88**, 47–50.

Nordenson, I., Sweins, A., and Beckman, L. (1981). Chromosomal aberrations in cultured human lymphocytes exposed to trivalent and pentavalent arsenic. *Scand. J. Work Environ. Health* **7**, 277–281.

Norseth, T. (1978). Health effects of nickel and chromium. *In* "Trace Metals, Exposure, and Health Effects" (di Ferrante, Ed.), pp. 135–146. Commission of the European Communities, Luxembourg.

Ott, M. G., Holder, B. B., and Gordo to arsenicals. *Arch. Environ. Hea*

Pershagen, G. (1981). The carcinogen

Pershagen, G. (1982). Arsenic and lur miological and experimental evide Institute, Stockholm.

Pershagen, G., Lind, B., and Björklu arsenic compounds. *Environ. Re:*

Pershagen, G., Wall, S., Taube, A., ar arsenic exposure and its relation 309.

Peto, R., Pike, M., Day, N., Gray, R. (1980). Guidelines for simple, se animal experiments. *In* "Long-Te Appraisal," pp. 311–426. Interna

Petres, J., Baron, D., and Hagedorn, ation: Cytogenic and biochemica

Pinto, S. S., Enterline, P. E., Hend relation to a measured arsenic tr

Rossman, T., Meyn, M., and Troll, V *Environ. Health Perspect.* 19, 2:

Rossman, T., Stone, D., Molina, M coli and Chinese hamster cells. *I*

Rudnay, P., and Borzsonyi, M. (19 *Magyar Onkologia* 25(2): 73–77.

Saffiotti, U., Cefis, F., and Kolb, L. genic carcinoma. *Cancer Res.* 2:

Saffiotti, U., Montesano, R., Sellakı tract carcinogenesis in hams benzo[*a*]pyrene and ferric oxide

Saffiotti, U., Montesano, R., Sellakı cinogenesis induced in hamsters *Natl. Cancer Inst.* 49(4), 1199–

Sjöstrand, B. (1977). Further develc and other elements in the occup Fund, Stockholm. (In Swedish)

Stenberg, U., and Alsberg, T. (1981 matic compounds adsorbed on

Stenbäck, F., and Rowland, J. (1979 mental, physiochemical and bio

Wicks, M., Archer, V., Auerbach, O as related to histological type o

PM3001119275

Source: https://www.industrydocuments.ucsf.edu/docs/jgxk0001

F. (1982). Effects of metals on sister
hamster V79-E cells. *ILO Occupat.*

O. (1978). Arsenic exposure and mor-
ter. *Brit. J. Ind. Med.* 35, 8–15.
nesis tests of two inorganic arsenicals.

um arsenite and arsanilic acid. *J. Agric.*

of 23 elements in the kidney, liver and
eden exposed to a number of elements

izo[*a*]pyrene from hamster lungs after
1, 1961–1964.
G. (1973). Respiratory tract tumors in
–1592.
rison of arsenic trioxide and calcium
ation. *Toxicol. Lett.* 12, 1–5.
(1977). Preliminary experimental study
. *Health Perspect.* 19, 191–196.
1980). Experimental study on carcino-
of rats by an intratracheal instillation.

Lung carcinoma induction in BD rats
ing pesticide mixture formerly used in

ronischen Arsenvergiftung. *Z. Haupt.*

lmonary carcinogenesis. *In* "Inhalation
, Eds.), pp. 321–351. AEC Symposium
Tennessee.
ratory cancer in man: An occupational

compounds in Salmonella typhimurium;

d occupational exposure to arsenic: A

erbach, O., Grundahl, R., and Wilson,
among members of copper-mining and
3.
s in bacteria. *Mutat. Res.* 31, 185–189.
ns in carcinogenesis. *Environ. Health*

, S. (1978). Occupational and environ-
I. Chromosomal aberrations in workers

iosomal aberrations in cultured human
ic. *Scand. J. Work Environ. Health* 7,

r "Trace Metals, Exposure, and Health
f the European Communities, Luxem-

Ott, M. G., Holder, B. B., and Gordon, H. L. (1974). Respiratory cancer and occupational exposure to arsenicals. *Arch. Environ. Health* 29, 250–255.

Pershagen, G. (1981). The carcinogenicity of arsenic. *Environ. Health Perspect.* 40, 93–100.

Pershagen, G. (1982). Arsenic and lung cancer, with special reference to interacting factors—Epidemiological and experimental evidence. Thesis at the Dept. of Environmental Hygiene, Karolinska Institute, Stockholm.

Pershagen, G., Lind, B., and Björklund, N.-E. (1982). Lung retention and toxicity of some inorganic arsenic compounds. *Environ. Res.* 29, 425–434.

Pershagen, G., Wall, S., Taube, A., and Linnman, L. (1981). On the interaction between occupational arsenic exposure and its relationship to lung cancer. *Scand. J. Work Environ. Health* 7, 302–309.

Peto, R., Pike, M., Day, N., Gray, R., Lee, P., Parish, S., Peto, J., Richards, S., and Wahrendorf, J. (1980). Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments. *In* "Long-Term and Short-Term Screening Assays for Carcinogens: A Critical Appraisal," pp. 311–426. International Agency for Research on Cancer, Lyon.

Petres, J., Baron, D., and Hagedorn, M. (1977). Effects of arsenic cell metabolism and cell proliferation: Cytogenic and biochemical studies. *Environ. Health Perspect.* 19, 223–227.

Pinto, S. S., Enterline, P. E., Henderson, V., and Varner, M. O. (1977). Mortality experience in relation to a measured arsenic trioxide exposure. *Environ. Health Perspect.* 19, 127–130.

Rossman, T., Meyn, M., and Troll, W. (1977). Effects of arsenite on DNA repair in Escherichia coli. *Environ. Health Perspect.* 19, 229–233.

Rossman, T., Stone, D., Molina, M., and Troll, W. (1980). Absence of arsenite mutagenicity in E. coli and Chinese hamster cells. *Environ. Mutagenesis* 2, 371–379.

Rudnay, P., and Borzsonyi, M. (1981). The tumorigenic effect of treatment with arsenic trioxide. *Magyar Onkologia* 25(2): 73–77.

Saffiotti, U., Cefis, F., and Kolb, L. H. (1968). A method for the experimental induction of bronchogenic carcinoma. *Cancer Res.* 28, 104–124.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G. (1972b). Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[*a*]pyrene and ferric oxide. *Cancer Res.* 32, 1073–1081.

Saffiotti, U., Montesano, R., Sellakumar, A. R., and Kaufman, D. G. (1972a). Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[*a*]pyrene and ferric oxide. *J. Natl. Cancer Inst.* 49(4), 1199–1204.

Sjöstrand, B. (1977). Further development of sensitive and accurate analytical methods for metals and other elements in the occupational environment. Report to the Swedish Work Environment Fund, Stockholm. (In Swedish)

Stenberg, U., and Alsberg, T. (1981). Vacuum sublimation and solvent extraction of polycyclic aromatic compounds adsorbed on carbonaceous material. *Anal. Chem.* 53, 2067–2072.

Stenbäck, F., and Rowland, J. (1979). Experimental respiratory carcinogenecis in hamsters: Environmental, physiochemical and biological aspects. *Oncology* 36, 63–71.

Wicks, M., Archer, V., Auerbach, O., and Kuschner, M. (1981). Arsenic exposure in a copper smelter as related to histological type of lung cancer. *Amer. J. Ind. Med.* 2, 25–31.

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